



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 33/53, 33/563, 33/567, C07K 1/00, 14/705		A1	(11) International Publication Number: WO 95/23970
			(43) International Publication Date: 8 September 1995 (08.09.95)
(21) International Application Number: PCT/US95/02426		(81) Designated States: AU, BR, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).	
(22) International Filing Date: 28 February 1995 (28.02.95)			
(30) Priority Data: 108789 1 March 1994 (01.03.94) IL 110142 28 June 1994 (28.06.94) IL		Published <i>With international search report.</i>	
(71) Applicant (for all designated States except US): YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; P.O. Box 95, 76100 Rehovot (IL).			
(71) Applicant (for BR only): RYCUS, Avigail [US/IL]; 16 Kipnis Street, 76305 Rehovot (IL).			
(72) Inventors; and (75) Inventors/Applicants (for US only): SHINITZKY, Meir [IL/IL]; 20 Derech Haganim Street, 49610 Kfar Shmaryahu (IL). DECKMANN, Michael [DE/FR]; 24, rue de Kreutzberger, F-68500 Guebwiller (FR).			
(74) Agents: OLIFF, James, A. et al.; Oliff & Berridge, P.O. Box 19928, Alexandria, VA 22320 (US).			
(54) Title: ASSAY FOR THE DIAGNOSIS OF SCHIZOPHRENIA			
(57) Abstract <p>An immunological assay for the diagnosis of schizophrenia in an individual is described. The assay comprises the following steps: (a) a blood sample, a platelet-containing fraction of a blood sample, or a fraction containing platelet-associated antibodies (PAA) shed from the platelets is obtained from an individual; (b) the sample is contacted with platelet antigens fixed to a solid support, and subsequently with an antibody detection system; and (c) the binding pattern of the PAA to the platelet antigens is determined and compared to the binding pattern of a sample obtained from a normal individual. A difference in patterns indicates that the individual has a high likelihood of having schizophrenia. The assay is capable of differentiating schizophrenia from dementia, as well as from Idiopathic Thrombocytopenia Purpura (ITP), an autoimmune disease directed against a platelet antigen.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

ASSAY FOR THE DIAGNOSIS OF SCHIZOPHRENIA

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to an assay for the diagnosis of schizophrenia.

5 The schizophrenic disorders, as defined by DSM-III (the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, 3rd edition, 1980) are mental disorders with a tendency towards chronicity which impairs functioning and which is characterized by
10 psychotic symptoms involving disturbances of thinking, feeling and behavior.

 Schizophrenia occurs worldwide. Although it is one of the most severe and prevalent mental disorders of well documented symptomatology and has been extensively
15 investigated over the past decades, the etiology of this disease is still an enigma.

 Hypotheses concerning the involvement of autoimmune elements in schizophrenia have been discussed for decades in the literature. Antibodies from sera of
20 some schizophrenic patients were shown to be able to react in vitro with brain tissue (DeLisi et al., 1986; Jankovic, 1984). However, these findings were contradictory and positive indications to the presence of autoantibodies in schizophrenic patients were obtained only in about 25% of
25 tested patients (De Lisi et al., 1985 and 1986).

 The existence of elevated levels of autoantibodies on blood platelets of both schizophrenic and demented patients has been reported recently by the inventors (Shinitzky et al., 1991). In that study, the level of
30 autoantibodies present on the surfaces of blood platelets (termed hereinafter "platelet-associated antibodies" (PAA)) of schizophrenic patients, patients with other mental disorders, e.g. Alzheimer-type and multi-infarct dementia (both treated with neuroleptics and untreated),
35 and normal control subjects was determined by the use of an enzyme-linked immunoassay based on color development after reaction with anti-human immunoglobulin (anti-human IgA, IgE, IgG and IgM antibodies) bound to horseradish

SUBSTITUTE SHEET (RULE 26)

peroxidase. The results of this study demonstrated that schizophrenic patients and demented patients had on the average about twice the level of PAA as compared to age-matched normal controls. Notwithstanding this statistically difference in the level of PAA between schizophrenic and demented patients versus normal controls, it was still too small to serve as basis for diagnosis of schizophrenia or dementia. Moreover, according to this study, similar results were obtained for schizophrenic and demented patients, not allowing identification of each of these disorders unequivocally.

It would be highly desirable to provide an assay for the specific diagnosis of schizophrenia that would identify schizophrenic but not demented patients, and would also not give false positive results with patients suffering from autoimmune diseases directed against blood platelets, e.g. idiopathic thrombocytopenia purpura (ITP).

This has now been achieved by the assay of the present invention.

SUMMARY OF THE INVENTION

In accordance with the present invention, PAA isolated from schizophrenic patients were contacted with proteins isolated from platelets (hereinafter "platelet antigens") and all cases developed a similar binding pattern. It was surprisingly found that the PAA from schizophrenic patients did not recognize and bind to well-known antigenic proteins from platelets involved in ITP, but recognized and bound to unknown platelet antigens which, on the other hand, were not recognized by the PAA from demented patients.

The present invention provides an assay for the diagnosis of schizophrenia in an individual, comprising the following steps:

- (a) obtaining a sample from said individual, being a blood sample, a platelet-containing fraction thereof, or a fraction containing platelet-associated antibodies (PAA) shed from the platelets;

(b) contacting said sample with platelet antigens fixed to a solid support and with an antibody detection system and;

(c) determining the pattern of binding of said PAA in said sample to their specific platelet antigen, a pattern different from normal individuals indicating that said individual has a high likelihood of having schizophrenia.

The sample of the individual to be tested may be a blood sample, e.g. serum, a platelet-containing fraction thereof or a PAA-containing fraction shed from the platelets. In a particular embodiment, the sample is platelet-rich plasma (PRP) obtained by known methods, e.g., treatment of blood with an anticoagulant, e.g. heparin or sodium citrate solution, and centrifugation. In another embodiment, the sample is a fraction containing PAA, obtained by centrifugation of PRP, incubation of the pelleted platelets with glycine-HCl buffer, pH 2.5-3.0 at room temperature and centrifugation, thus obtaining a PAA-containing supernatant.

The platelet antigens are obtained e.g. by disintegration of platelets derived from PRP and separated according to their molecular weights by polyacrylamide gel electrophoresis (PAGE) and transfer to a solid support, e.g., nitrocellulose.

The antibody detection systems to detect PAA levels comprises anti-human immunoglobulin (anti-hIg) antibody, or a fragment thereof, linked to a marker. Such a marker may, for example, be a radioactive group, a fluorescent group, an enzyme that can catalyze a reaction yielding a detectable product such as, for example, horseradish peroxidase (HRP) and alkaline phosphatase, a biotin group that can be detected by avidin, etc.

In a particular embodiment the antibody is rabbit anti-human IgG (anti-hIgG) covalently linked to HRP and ortho-phenyl-enediamine is the reagent used to detect bound HRP.

SUBSTITUTE SHEET (RULE 26)

By another embodiment, a fragment of IgG is used which is Fc-depleted, such as an Fab or an F(ab')₂ fragment or a part thereof which contains the fragment's binding domain, or a single-chain antibody, or the like. In case such single domain antibodies are used, the degree of binding of the antibodies to PAA can be determined by the use of a second antibody directed against said single domain antibody, which second antibody is bound to a marker as above.

By a still further embodiment, a solid support having immobilized thereon a PAA target antigen(s) specific for schizophrenia, i.e., the antigen(s) to which the PAA of schizophrenic patients are directed, is used. The PAA target antigen(s) specific for schizophrenia will be isolated from the antigenic proteins recognized by the PAA from schizophrenic patients, purified and characterized. In accordance with a first modification of this embodiment, the platelets are contacted with the support and following an incubation period, the immobilized platelets are reacted with the anti-hIg antibodies, which are preferably labelled and the number of bound PAA can thus be determined. By a second modification of this embodiment, the platelets are first treated to shed their PAA, the PAA-containing fraction is then reacted with the support and following incubation and washing, the supports are reacted with the anti-hIg antibodies which are preferably labelled, and the number of bound PAA is thus determined.

The present invention also provides a kit useful in the above assay. The kit of the invention comprises an anti-hIg antibody or a fragment thereof and a support comprising a sole antigen or a plurality of platelet antigens reactive with the PAA immobilized onto the support. According to one embodiment, the antibodies in the kit are conjugated to a detectable marker. In accordance with another embodiment, when fragments of antibodies are used, the kit comprises also a second type of antibodies directed against said single domain

antibodies, which second type of antibodies are in turn conjugated to a detectable marker. In accordance with a further embodiment, the antibodies are immobilized onto a support and the kit comprises such a support. The kit according to all above embodiments may also comprise the various reagents required for carrying out the assay.

The invention will now be illustrated in the following non-limiting description of specific embodiments and accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-B show the pattern of binding by Western blot of PAA obtained from 10 normal control subjects (A) and 8 schizophrenic patients (B) to platelet antigens in a nitrocellulose support, separated by 10% acrylamide gel, as determined by HRP-labelled rabbit anti-human IgG.

Figs. 2A-C show the pattern of binding by Western blot of PAA from 4 schizophrenic patients (A), 4 normal control subjects (B) and 4 demented patients (C) to platelet antigens in a nitrocellulose support, separated by 10% acrylamide gel, as determined by HRP-labelled rabbit anti-human IgG.

Fig. 3 shows the pattern of binding by Western blot of PAA from 10 schizophrenic patients (lanes 1-10) to platelet antigens on a nitrocellulose support, separated by a 7,5-15% acrylamide gradient gel, as determined by HRP-labelled rabbit anti-human IgG.

EXAMPLES

General Procedures

(i) Patients and control subjects

Chronic schizophrenic and demented patients, hospitalized in mental or geriatric hospitals in Israel participated in this study. They consisted both of patients treated with various neuroleptics and newly diagnosed and untreated patients. All patients were free of any immunological or allergic disorder. The control group consisted of healthy volunteers who had no history of psychiatric disorders or any chronic disease.

(ii) Determination of PAA levels

Venous blood (20 ml) was drawn in the morning using sodium citrate solution (sodium citrate - 2.2%, citric acid - 0.73%, dextrose monohydrate - 2.45%) as an anticoagulant. Platelet-rich plasma (PRP) was obtained from full blood by slow centrifugation (100xg for 15 min) at room temperature (20-24°C), and platelets were scored microscopically.

PAA levels were determined either with rabbit anti-human IgG antibodies linked to HRP (as in Shinitzky et al., 1991) or with a Fab fragment of this antibody. Enzyme-linked immunoassay (ELI) based on a color development after binding of the antibody or Fab fragment thereof, was used.

For the preparation of the anti-human IgG Fab fragment bound to HRP, activated plastic beads (Immunotip, U.S.A. Scientific Plastics) were coupled with papain as follows: 1 mg papain (Worthington, U.S.A.) was mixed in 1 ml of 0.2M sodium cyanoborohydride (Fluka, U.S.A.) and was incubated with a single plastic bead for 5-10 minutes, and then washed extensively with phosphate-buffered saline (PBS). Horseradish peroxidase-conjugated rabbit anti-human IgG (BioMakor, Israel; 740 µg in 40 µl) was incubated with gentle shaking with the papain-conjugated bead for 5 hours at 37°C and then passed through a protein A column (Pierce). The Fab fragment of the treated antibodies were collected while washing with 15 ml 10 mM Tris buffer, pH=7.4.

For the ELI procedure (Leporrier et al., 1979) 300 µl PRP were centrifuged and the pellet was resuspended in 1 ml of PBS pH 7.2. This step was repeated 3 times. The pellet was then resuspended in 0.15 ml PBS containing either rabbit anti-human IgG linked to HRP or its Fab fragment linked to HRP, and incubated for 30 min at 37°C. After 4 washings with PBS at 4°C, the platelet suspension was incubated with freshly prepared substrate reagent (19.8 ml PBS - 0.2 ml methanol containing 2 mg ortho-phenylenediamine + 3 µl H₂O₂ 30%) for 1 hr at 37°C. The reaction was terminated by adding 0.1 ml of 6N sulfuric

acid. After centrifugation, the O.D. was read at 480 nm. After background subtraction, the O.D. was calculated for 10^8 platelets per ml (Shinitzky et al., 1991).

(iii) Isolation of PAA from platelets

5 PRP (4 to 15 ml) was centrifuged (2000xg for 15 min) at room temperature. The supernatant was discarded and the pellet was resuspended in 5 ml PBS, pH 7.2. This step was repeated three times. For the removal of PAA from the pelleted platelets, the pH was lowered by
10 incubation with 2 ml 150 mM glycine-HCl buffer, pH 2.5, for 5 min at room temperature. The platelets were pelleted by centrifugation (2000xg for 15 min) and the PAA-containing supernatant was collected and immediately neutralized to pH 6.5 with 2 ml 200 mM phosphate buffer,
15 pH 7.5.

(iv) Preparation of platelet antigens by polyacrylamide gel electrophoresis (PAGE)

Platelets were derived from PRP by 3 washings with PBS and separated by PAGE according to their molecular
20 weights as described (Laemmli U.K., Nature, 227, 680 (1970)). Depending on the molecular weight of interest, 5%, 10% or 12.5% acrylamide concentrations were used. Alternatively, a 7.5-15% acrylamide gradient gel was used, leading to a better resolution than homogeneous acrylamide
25 concentration.

(v) Western blot

Before the electrophoretic transfer, a small vertical piece was sliced from the gel and stained with Coomassie Blue to assess the success of the gel
30 electrophoresis. For the electrophoretic protein transfer, the gel was placed between sheets of nitrocellulose, Whatmann filter paper No. 4 and pads as holders. The transfer was performed in a Western blot cell (BioRad) filled with transfer buffer (0.025M Tris,
35 0.192M glycine, 0.1% SDS, 20% methanol, pH 8.3), by 300 mA (milli Ampère) for one hour at room temperature and without cooling.

To monitor the efficiency of the protein transfer, the nitrocellulose sheet was stained with the reversibly-binding dye Poinceau S. After Removal of the Poinceau S stain with double distilled water, the nitrocellulose sheet was air-dried, cut into 0.3 cm wide strips and stored dry at room temperature until use.

(vi) Western blot analysis

Platelet proteins from control subjects (blood group O) were separated by 10% PAGE and blotted onto nitrocellulose as described in (v) above. All incubation steps were performed at room temperature and under constant shaking (Bellco Rocking Table, speed setting 4) in BioRad Incubation Trays. The sample strips and positive control strip (dotted with 0.002 ml human serum) were blocked with 1 ml blocking buffer (5% milk powder dissolved in incubation buffer [60 mM citric acid, 90 mM Na_2HPO_4 , 200 mM NaCl, pH 7.7] filtered before use) for 30 min. The blocking buffer was then carefully removed (with a Pasteur pipette) and the strips were supplemented with 1 ml incubation buffer. PAA (0.2 ml) or serum (0.1 ml) was then added to the strips as a sample and incubated for 14 hours. After the incubation, the strips were washed 3 times with 1 ml incubation buffer for 15 min. and one time with 1 ml peroxidase buffer (200 mM Tris, 150 mM KCl, 0.3% Triton X-100, 10 mM phenol, 2 mM CaCl_2 , filtered before use) for 15 min. The strips were incubated with rabbit anti-human IgG covalently linked to peroxidase (1:1000 diluted) in 1 ml peroxidase buffer for 2 hours. After this incubation, the strips were washed 3 times with 1 ml peroxidase buffer for 15 min. and one time with 1 ml PBS for 15 min. Bound peroxidase was visualized by incubating the strips with 1 ml Color Reagent (12 mg chloronaphthol, 4 ml methanol, 20 ml PBS, 0.005 ml 30% H_2O_2) for 30-45 min. The color reaction was stopped by washing the strips with double distilled water.

EXAMPLE 1

PAA from 10 blood donors (Fig. 1A) and from 8 chronic schizophrenic patients (Fig. 1B) were incubated

with normal human platelet proteins, separated by gel electrophoresis and blotted onto nitrocellulose as described above. Visual detection of bound PAA was carried out as described above. The results are shown in Fig. 1, in which the following molecular weight markers (BioRad) were used: lysozyme - 14.4 kD; trypsin inhibitor - 21.5 kD;; carbonic anhydrase - 31 kD; ovalbumin - 45 kD; serum albumin - 66.2 kD and phosphorylase B - 97.4 kD. No bands were observed in any of the 10 examples of PAA from normal donors (Fig. 1A), while in all 8 examples of PAA from chronic schizophrenic patients a series of bands was detected (Fig. 1B).

The same pattern was surprisingly observed in all 8 examples of schizophrenic patients. The following relative molecular weights could be attributed to the identified proteins by molecular weight estimation: 35 kD; 43 kD; 47 kD; 50 kD; 64 kD; 68 kD; 97 kD; 120 kD. These molecular weights do not fit to the well known proteins involved in Idiopathic Thrombocytopenia Purpura (ITP), an autoimmune disease directed against a structural platelet antigen. The proteins involved in Idiopathic Thrombocytopenia Purpura (ITP) are Proteins I b (α -unit, 128-141 kD; β -unit, 22 kD), II b (α -unit, 132 kD; β -unit, 23 kD) and III a (95 kD) (Williams W.J. et al., eds., Hematology, 4th ed., 1990, McGraw-Hill Inc., pp. 1189 and 1382). It should be noted that although a 120 kD antigen is recognized by the PAA from schizophrenic patients no 22 kD antigen is recognized by them, thus indicating that the PAA from schizophrenic patients identified according to the invention are not the same entities involved in ITP. Moreover, all the schizophrenic patients tested were free of ITP.

EXAMPLE 2

Another experiment was carried out with 4 normal blood donors (Fig. 2B), 4 chronic schizophrenic patients (Fig. 2A) and 4 patients with Alzheimer-type or multi-infarct dementia, disorders that are also known to have elevated PAA levels (Shinitzky et al., 1991). As shown in

Fig. 2, the PAA of the demented patients (Fig. 1C) do not give any reaction in the Western blot while the Western blot of the 4 schizophrenic patients shows a common pattern for all 4 patients (Fig. 1A).

5 EXAMPLE 3

 An experiment was carried out with 10 schizophrenic patients using a 7,5-15% acrylamide gradient gel. The results are shown in Fig. 3, where the Western blot of the PAA of the 10 schizophrenic patients shows a
10 common pattern (lanes 1-10). On the left side are shown the positions of the molecular weight markers (BioRad). Lane 11 shows Coomassie Blue stain of platelet proteins.

 The following relative molecular weights could be attributed to the proteins identified in lanes 1-10, by
15 molecular weight estimation: 17, 18, 21, 24, 26, 35, 40-43, 47, 50, 55, 68, 76, 82, 95-97 (two bands), 105, 120-130, 165-175.

REFERENCES

 DeLisi, L.E. and Crow, T.J., 1986. Is
20 schizophrenia a viral or immunological disorder? Psychiatr. Clin. North. Am. 9: 115-132.

 DeLisi, L.E. Weber, R.J. and Pert, C.B., 1985. Are there antibodies against brain in sera from schizophrenic patients? Review and Perspectus. Biol.
25 Psychiatry, 20: 94-119.

 Fudenberg, H.H., Whitten, H.D., Merler, E. and Farmati, O., 1983. Is schizophrenia an autoimmunologic receptor disorder? Med. Hypothes., 12: 85-93.

 Jankovic, B.D., 1984. From Immunoneurology to
30 Immunopsychiatry. Neuromodulating activity of anti-brain antibodies. Int. Rev. Neurobiol., 26: 249-314.

 Leporrier, M., Dighiero, G., Auzemery, M. and Binet, J.L., 1979. Detection and quantification of platelet-bound antibodies with immunoperoxidase. Br. J.
35 Haematol., 42: 605-611.

 Shinitzky, M., Deckmann, M., Kessler, A., Sirota, P., Rabbs, A. and Elizur, A., 1991. Platelet autoantibodies in dementia and schizophrenia - possible

implication for mental disorders. An. N.Y. Acad. Sci.,
621: 205-217.

SUBSTITUTE SHEET (RULE 26)

CLAIMS

1. An assay for the diagnosis of schizophrenia in an individual, comprising the following steps:

5 (a) obtaining a sample from said individual, being a blood sample, a platelet-containing fraction thereof, or a fraction containing platelet-associated antibodies (PAA) shed from the platelets;

10 (b) contacting said sample with platelet antigens fixed to a solid support and with an antibody detection system, and;

15 (c) determining the pattern of binding of said PAA in said sample to their specific platelet antigen, a pattern different from normal individuals indicating that said individual has a high likelihood of having schizophrenia.

2. An assay according to Claim 1, wherein said sample is a platelet-containing blood fraction.

20 3. An assay according to Claim 2, wherein said sample is platelet-rich plasma.

4. An assay according to Claim 1, wherein said sample is a PAA-containing fraction.

25 5. An assay according to Claims 1-4, wherein said platelet antigens are a plurality of antigenic proteins obtained from platelets.

30 6. An assay according to Claim 5, wherein said platelet antigens are separated by polyacrylamide gel electrophoresis and are immobilized onto a nitrocellulose support.

7. An assay according to Claim 6, wherein a 7,5-15% acrylamide gradient gel is used.

35 8. An assay according to any one of Claims 1-4, wherein said platelet antigens comprise a sole antigen to which the PAA of schizophrenic patients are directed.

9. An assay according to any one of Claims 1-8, wherein said antibody detection system is an anti-human immunoglobulin antibody or a fragment thereof.

SUBSTITUTE SHEET (RULE 26)

10. An assay according to Claim 9, wherein said anti-human immunoglobulin antibody is an anti-hIgG antibody or a fragment thereof.

5 11. An assay according to Claim 10, wherein said anti-hIg antibody fragment is an Fab fragment of an anti-hIg antibody.

12. An assay according to Claim 10, wherein said anti-hIg antibody fragment is an F(ab')₂ fragment of an anti-hIg antibody.

10 13. An assay according to any one of Claims 1-12, wherein the anti-hIg antibody is conjugated to a marker.

14. An assay according to Claim 13, wherein said marker is an enzyme capable of catalyzing a reaction which yields a detectable product.

15 15. An assay according to any one of Claims 11-12, wherein the degree of binding is determined by the use of a second antibody directed against said single domain antibody fragment, which second antibody is bound to a detectable marker.

20 16. An assay for the diagnosis of schizophrenia in an individual, comprising:

(a) obtaining a sample from said individual, being a blood sample, a platelet-containing fraction thereof or a
25 fraction containing the platelet-associated antibodies (PAA) shed from the platelets;

(b) contacting said sample with a solid support having thereof an antigen to
30 which the PAA of schizophrenia patients are directed; and

(c) contacting said support with anti-hIg antibody and determining binding of said antibody to the support.

35 17. A kit for use in any one of the assays of claims 1-16.

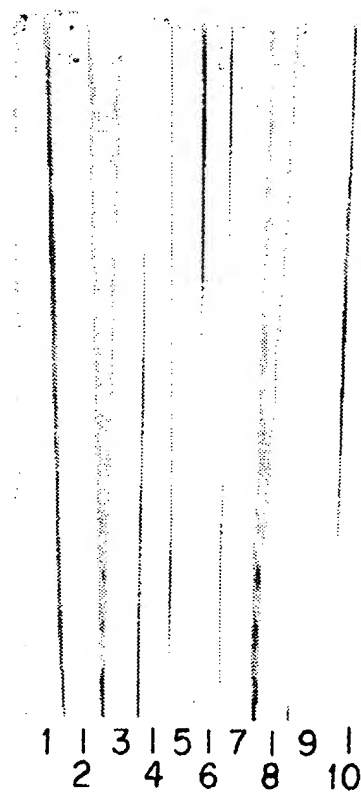


FIG. 1A

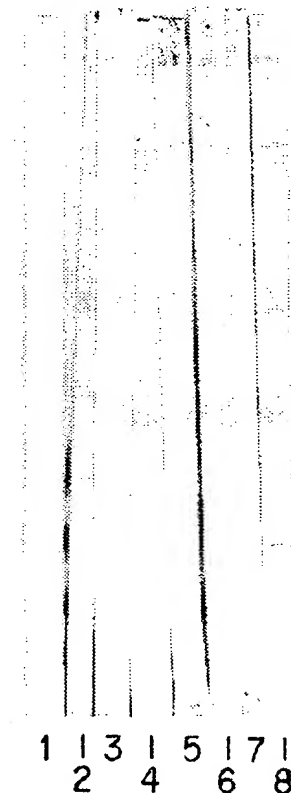


FIG. 1B

SUBSTITUTE SHEET (RULE 26)

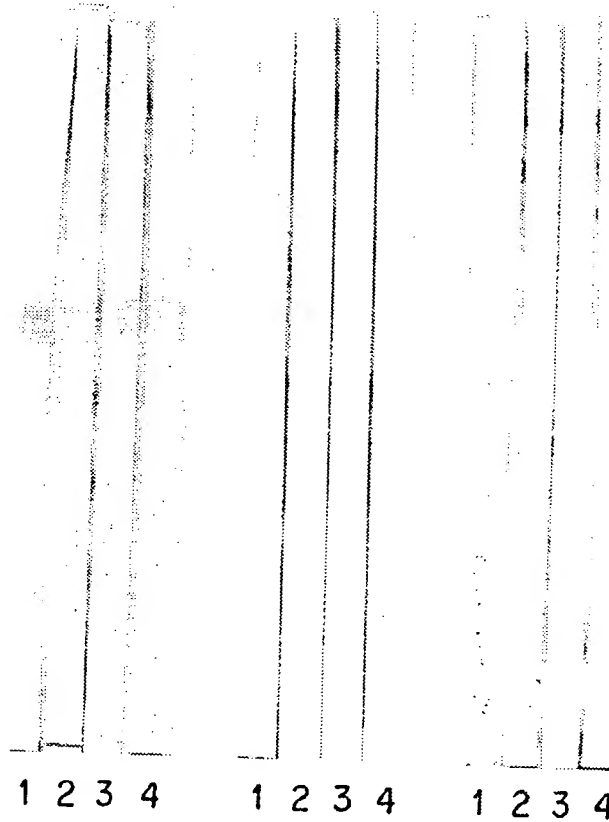
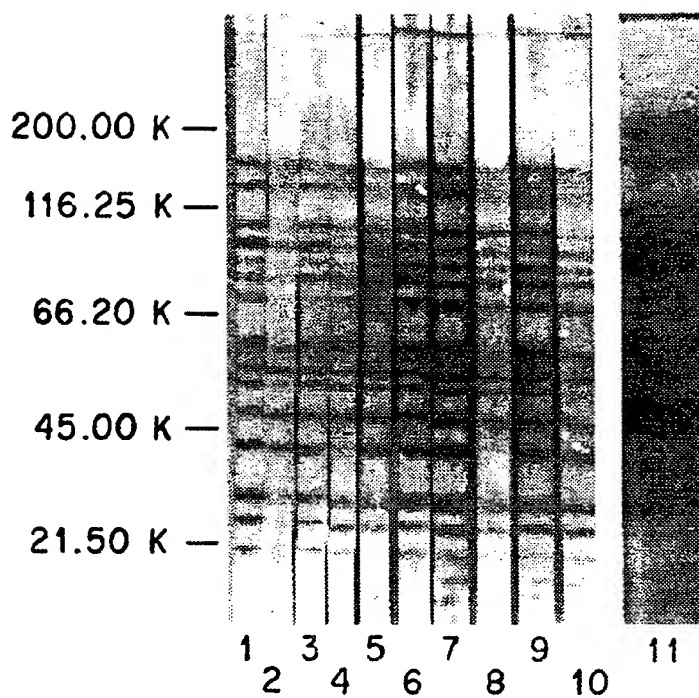


FIG. 2A FIG. 2B FIG. 2C

SUBSTITUTE SHEET (RULE 26)

3/3

**FIG. 3**

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02426**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, APS, JPO

search terms: schizophreni?, platelet, antibody, autoantibody

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANNALS NEW YORK ACADEMY OF SCIENCES, Volume 621, issued 01 July 1991, M. Shinitzky et al., "Platelet Autoantibodies in Dementia and Schizophrenia -- Possible Implications for Mental Disorders," pages 205-217, especially pages 206-207, 214.	1-17
Y	PSYCHOBIOLOGY, Volume 21, No. 4, issued April 1993, A. Kessler et al., "Platelets from schizophrenic patients bear autoimmune antibodies that inhibit dopamine uptake", pages 299-306, especially pages 300-301, 304-305.	1-17
Y,P	LANCET, Volume 344, No. 8914, issued 02 July 1994, J. Levine et al., "Treatment of schizophrenia with an immunosuppressant", pages 59-60, see entire article.	1-17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

11 MAY 1995

Date of mailing of the international search report

22 MAY 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

CAROL A. SPIEGEL

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/02426

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

G01N 33/53, 33/563, 33/567; C07K 1/00, 14/705

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/7.21, 7.92, 7.95; 436/503, 512, 518; 530/387.1, 389.1, 389.6

B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

435/7.21, 7.9, 7.92, 7.93, 7.94, 7.95, 810, 975; 436/503, 512, 518, 808; 530/387.1, 389.1, 389.6, 806, 809;
424/153.1